

SEQUENCE LISTING

<110> Leppla, Stephen H.
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Liu, Shi-Hui
Osorio, Manuel
The Government of the United States of America
as represented by The Secretary of the
Department of Health and Human Services

<120> Activation of Recombinant Diphtheria Toxin Fusion
Proteins by Specific Proteases Highly Expressed on the
Surface of Tumor Cells

<130> 015280-478100US

<140> US 10/554,076
<141> 2005-10-21

<150> US 60/468,577
<151> 2003-05-06

<150> WO PCT/US04/14306
<151> 2004-05-06

<160> 30

<170> PatentIn Ver. 2.1

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<213> Artificial Sequence

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<223> Description of Artificial Sequence:DTGM-L1
      DT-GMCSF fusion protein in which native furin
      recognition cleavage site replaced by matrix
      metalloproteinase (MMP) recognition cleavage site

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<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence:DTGM-L2
DT-GMCSF fusion protein in which native furin
recognition cleavage site replaced by matrix
metalloproteinase (MMP) recognition cleavage site

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<211> 1560

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTGM-U2 DT-GMCSF fusion
protein in which native furin recognition cleavage site
replaced by urokinase-type plasminogen activator (uPA)
recognition cleavage site

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<211> 1560

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTGM-U3 DT-GMCSF fusion protein in which native furin recognition cleavage site replaced by urokinase-type plasminogen activator (uPA) recognition cleavage site

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<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:DTEGF-L1 DT-EGF
fusion protein in which native furin recognition
cleavage site replaced by matrix metalloproteinase
(MMP) recognition cleavage site

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<211> 1341

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:DTEGF-L2 DT-EGF
fusion protein in which native furin recognition
cleavage site replaced by matrix metalloproteinase
(MMP) recognition cleavage site

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<211> 1335

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: DTEGF-U2 DT-EGF fusion protein in which native furin recognition cleavage site replaced by urokinase-type plasminogen activator (uPA) recognition cleavage site

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cacgatgggt actgtctaca tgacggcgct tgtatgtata ttgaggctct agacaagtac 1260
gcgtgtaatt gcgttggttg ctacatcggt gagcgtgtgc agtatcgaga tctgaaatgg 1320
tggaactta gataa                                     1335

```

<210> 9

<211> 1335

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: DTEGF-U3 DT-EGF fusion protein in which native furin recognition cleavage site replaced by urokinase-type plasminogen activator (uPA) recognition cleavage site

<400> 9

```

atgggcgcgg acgacgtcgt cgactcttct aaatcttttg tgatggaaaa cttttcttcg 60
taccacggga ctaaacctgg ttatgtagat tccattcaaa aaggtataca aaagccaaaa 120
tctgggtacac aaggaaatta tgacgatgat tggaaagggg tttatagtag cgacaataaa 180
tacgacgctg cgggatactc tgtagataat gaaaaccgcg tctctggaaa agctggaggc 240

```

gtggtcaaag	tgacgtatcc	aggactgacg	aaggttctcg	cactaaaagt	ggataatgcc	300
gaaactatta	agaaagagtt	aggtttaagt	ctcactgaac	cgttgatgga	gcaagtcgga	360
acggaagagt	ttatcaaaaag	gttcggtgat	ggtgcttcgc	gtgtagtgt	cagccttccc	420
ttcgctgagg	ggagttctag	cgttgaatat	attaataact	gggaacaggc	gaaagcggtta	480
agcgtagaac	ttgagattaa	ttttgaaacc	cgtggaaaac	gtggccaaga	tgcgatgtat	540
gagtatatgg	ctcaagcctg	tgcaggaaat	ggaagtggaa	aatcagcagg	tagctcattg	600
tcatgcataa	atcttgattg	ggatgtcata	agggataaaa	ctaagacaaa	gatagagtct	660
ttgaaagagc	atggccctat	caaaaataaa	atgagcgaaa	gtcccaataa	aacagtatct	720
gaggaaaaag	ctaaacaata	cctagaagaa	tttcatcaaa	cggcattaga	gcatcctgaa	780
ttgtcagaac	ttaaaaccgt	tactgggacc	aatcctgtat	tcgctggggc	taactatgcy	840
gcgtgggagc	taaacgttgc	gcaagttatc	gatagcgaaa	cagctgataa	tttggaaaaag	900
acaactgctg	ctctttcgtat	acttcctggt	atcggtagcg	taatgggcat	tgcagacggt	960
gccgttcacc	acaatacaga	agagatagtg	gcacaatcaa	tagctttatc	gtctttaatg	1020
gttgctcaag	ctattccatt	ggtaggagag	ctagttgata	ttggtttcgc	tgcataataat	1080
tttgtagaga	gtattatcaa	tttatttcaa	gtagttcata	attcgtataa	tcgtcccgcg	1140
tattctccc	ggcataaaaac	gaggcctcat	atgaattccg	atagcgagtg	tcctctgagt	1200
cacgatgggt	actgtctaca	tgacggcgct	tgtatgtata	ttgaggctct	agacaagtac	1260
gcgtgtaatt	gcgttggttg	ctacatcggt	gagcgctgtc	agtatcgaga	tctgaaatgg	1320
tgggaactta	gataa					1335

<210> 10

<211> 1581

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTIL2-L1 DT-IL2
fusion protein in which native furin recognition
cleavage site replaced by matrix metalloproteinase
(MMP) recognition cleavage site

<400> 10

atgggcgccg	acgacgtcgt	cgactcttct	aaatcttttg	tgatggaaaa	cttttcttcg	60
taccacggga	ctaaacctgg	ttatgtagat	tccattcaaa	aaggtataca	aaagccaaaa	120
tctggtacac	aaggaaatta	tgacgatgat	tggaaagggg	tttatagtag	cgacaataaa	180
tacgacgctg	cgggatactc	tgtagataat	gaaaacccgc	tctctggaaa	agctggaggc	240
gtggtcaaag	tgacgtatcc	aggactgacg	aaggttctcg	cactaaaagt	ggataatgcc	300
gaaactatta	agaaagagtt	aggtttaagt	ctcactgaac	cgttgatgga	gcaagtcgga	360
acggaagagt	ttatcaaaaag	gttcggtgat	ggtgcttcgc	gtgtagtgt	cagccttccc	420
ttcgctgagg	ggagttctag	cgttgaatat	attaataact	gggaacaggc	gaaagcggtta	480
agcgtagaac	ttgagattaa	ttttgaaacc	cgtggaaaac	gtggccaaga	tgcgatgtat	540
gagtatatgg	ctcaagcctg	tgcaggaaat	ggaccattag	gaatgttgag	tcaaggtagc	600
tcattgtcat	gcataaatct	tgattgggat	gtcataaggg	ataaaactaa	gacaaagata	660
gagtccttga	aagagcatgg	ccctatcaaa	aataaaatga	gcgaaagtcc	caataaaaca	720
gtatctgagg	aaaaagctaa	acaataccta	gaagaatttc	atcaaacggc	attagagcat	780
cctgaattgt	cagaacttaa	aaccgttact	gggaccaatc	ctgtattcgc	tggggctaac	840
tatgcggcgt	gggcagtaaa	cgttgcgcaa	gttatcgata	gcgaaacagc	tgataatttg	900
gaaaagacaa	ctgtgctct	ttcgatactt	cctggtagtg	gtagcgtaat	gggcattgca	960
gacggtgccg	ttcaccacaa	tacagaagag	atagtgccac	aatcaatagc	tttatcgctc	1020
ttaatgggtg	ctcaagctat	tccattggta	ggagagctag	ttgatattgg	tttcgctgca	1080
tataattttg	tagagagtag	tatcaattta	tttcaagtag	ttcataattc	gtataatcgt	1140
cccgcgtatt	ctcccgggca	taaaacgagg	cctcatatgg	cacctacttc	aagttctaca	1200
aagaaaacac	agctacaact	ggagcattta	ctgctggatt	tacagatgat	tttgaatgga	1260
attaataatt	acaagaatcc	caaactcacc	aggatgctca	catttaagtt	ttacatgccc	1320
aagaaggcca	cagaactgaa	acatcttcag	tgtctagaag	aagaactcaa	acctctggag	1380
gaagtgctaa	atttagctca	aagcaaaaac	tttactttaa	gaccagggga	cttaatcagc	1440
aatatcaacg	taatagttct	ggaactaaag	ggatctgaaa	caacattcat	gtgtgaatat	1500
gctgatgaga	cagcaacctat	tgtagaattt	ctgaacagat	ggattacctt	ttgtcaaagc	1560
atcatctcaa	cactgacttg	a				1581

<210> 11
 <211> 1581
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTIL2-L2 DT-IL2
 fusion protein in which native furin recognition
 cleavage site replaced by matrix metalloproteinase
 (MMP) recognition cleavage site

<400> 11

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atggg'gcgcg acgacgctcg cgactcttct aaatcttttg tgatggaaaa cttttcttcg 60
taccacggga ctaaacctgg ttatgtagat tccattcaaa aaggtataca aaagccaaaa 120
tctggtacac aaggaaatta tgacgatgat tggaaagggt tttatagtag cgacaataaa 180
tacgacgctg cgggatactc tgtagataat gaaaacccgc tctctggaaa agctggaggc 240
gtggtcaaaag tgacgtatcc aggactgacg aaggttctcg cactaaaagt ggataatgcc 300
gaaactatta agaaagagtt aggtttaagt ctcaactgaac cgttgatgga gcaagtcgga 360
acggaagagt ttatcaaaaag gttcgggtgat ggtgcttcgc gtgtagtgct cagccttccc 420
ttcgctgagg ggagttctag cgttgaatat attaataact gggaacaggc gaaagcgta 480
agcgtagaac ttgagattaa ttttgaaacc cgtggaaaac gtggccaaga tgcgatgtat 540
gagtatatgg ctcaagcctg tgcaggaaat ggaccattag gattatgggc acaaggtagc 600
tcattgtcat gcataaatct tgattgggat gtcataaggg ataaaaactaa gacaaagata 660
gagtctttga aagagcatgg ccctatcaaa aataaaatga gcgaaagtcc caataaaaca 720
gtatctgagg aaaaagctaa acaataccta gaagaatttc atcaaacggc attagagcat 780
cctgaattgt cagaacttaa aaccgttact gggaccaatc ctgtattcgc tggggctaac 840
tatgcggcgt gggcagtaaa cgttgcgcaa gttatcgata gcgaaacagc tgataatttg 900
gaaaagacaa ctgctgctct ttcgatactt cctgggtatcg gtacgtaat gggcattgca 960
gacggtgccg ttcaccacaa tacagaagag atagtggcac aatcaatagc tttatcgtct 1020
ttaatggttg ctcaagctat tccattggta ggagagctag ttgatattgg tttcgctgca 1080
tataattttg tagagagtat tatcaattta tttcaagtag ttcataattc gtataatcgt 1140
cccgcgattt ctcccgggca taaaacgagg cctcatatgg cacctacttc aagttctaca 1200
aagaaaacac agctacaact ggagcattta ctgctggatt tacagatgat tttgaatgga 1260
attaataatt acaagaatcc caaactcacc aggatgctca catttaagtt ttacatgccc 1320
aagaaggcca cagaactgaa acatcttcag tgtctagaag aagaactcaa acctctggag 1380
gaagtgctaa atttagctca aagcaaaaac tttcacttaa gaccagggga cttaatcagc 1440
aatatcaacg taatagttct ggaactaaag ggatctgaaa caacattcat gtgtgaatat 1500
gctgatgaga cagcaacat tgtagaattt ctgaacagat ggattacctt ttgtcaaagc 1560
atcatctcaa cactgacttg a                                     1581

```

<210> 12
 <211> 1575
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTIL2-U2 DT-IL2 fusion
 protein in which native furin recognition cleavage site
 replaced by urokinase-type plasminogen activator (uPA)
 recognition cleavage site

<400> 12

```

atggg'gcgcg acgacgctcg cgactcttct aaatcttttg tgatggaaaa cttttcttcg 60
taccacggga ctaaacctgg ttatgtagat tccattcaaa aaggtataca aaagccaaaa 120
tctggtacac aaggaaatta tgacgatgat tggaaagggt tttatagtag cgacaataaa 180
tacgacgctg cgggatactc tgtagataat gaaaacccgc tctctggaaa agctggaggc 240
gtggtcaaaag tgacgtatcc aggactgacg aaggttctcg cactaaaagt ggataatgcc 300
gaaactatta agaaagagtt aggtttaagt ctcaactgaac cgttgatgga gcaagtcgga 360
acggaagagt ttatcaaaaag gttcgggtgat ggtgcttcgc gtgtagtgct cagccttccc 420
ttcgctgagg ggagttctag cgttgaatat attaataact gggaacaggc gaaagcgta 480

```



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agcgtagaac ttgagattaa ttttgaaacc cgtggaaaac gtggccaaga tgcgatgtat 540
gagtatatgg ctcaagcctg tgcaggaaat ggaagtggaa gatcagcagg tagctcattg 600
tcatgcataa atcttgattg ggatgtcata agggataaaa ctaagacaaa gatagagtct 660
ttgaaagagc atggccctat caaaaataaa atgagcgaaa gtcccaataa aacagtatct 720
gaggaaaaag ctaaacaata cctagaagaa tttcatcaaa cggcattaga gcatcctgaa 780
ttgtcagaac ttaaaaccgt tactgggacc aatcctgtat tcgctggggc taactatgcg 840
gcgtgggcag taaacgttgc gcaagttatc gatagcgaaa cagctgataa tttggaaaag 900
acaactgctg ctctttcgat acttcctggg atcggtagcg taatgggcat tgcagacggg 960
gccgttcacc acaatacaga agagatagtg gcacaatcaa tagctttatc gtctttaatg 1020
gttgctcaag ctattccatt ggtaggagag ctagttgata ttggtttcgc tgcataata 1080
ttttagagaa gtattatcaa tttatttcaa gtagttcata attcgtataa tcgtcccgcg 1140
tattctcccg ggcataaaaac gaggcctcat atggcaccta cttcaagttc tacaagaaa 1200
acacagctac aactggagca tttactgctg gatttacaga tgattttgaa tggaaatta 1260
aattacaaga atcccaact caccaggatg ctcacattta agttttacat gcccaagaag 1320
gccacagaac tgaaacatct tcagtgtcta gaagaagaac tcaaacctct ggaggaagt 1380
ctaaatttag ctcaaagcaa aaactttcac ttaagacca gggacttaat cagcaatatt 1440
aacgtaatag ttctggaact aaagggatct gaaacaacat tcattgtgtg atatgctgat 1500
gagacagcaa ccattgtaga atttctgaac agatggatta ctttttgtca aagcatcatt 1560
tcaacactga cttga 1575

```

<210> 13

<211> 1575

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DTIL2-U3 DT-IL2 fusion protein in which native furin recognition cleavage site replaced by urokinase-type plasminogen activator (uPA) recognition cleavage site

<400> 13

```

atgggcgcgc acgacgtcgt cgactcttct aaatcttttg tgatggaaaa cttttcttcg 60
taccacggga ctaaaccctg ttatgtagat tccattcaaa aaggtataca aaagccaaaa 120
tctggtagac aaggaaatta tgacgatgat tggaaagggt tttatagtac cgacaataaa 180
tacgacgctg cgggatactc tgtagataat gaaaaccgcg tctctggaaa agctggaggc 240
gtggtcaaag tgacgtatcc aggactgacg aaggttctcg cactaaaagt ggataatgcc 300
gaaactatta agaaagagtt aggtttaagt ctactgaac cgttgatgga gcaagtcgga 360
acggaagagt ttatcaaaag gttcgggtgat ggtgcttcgc gtgtagtgtc cagccttccc 420
ttcgctgagg ggagttctag cgttgaatat attaataact gggaacaggc gaaagcgtta 480
agcgtagaac ttgagattaa ttttgaaacc cgtggaaaac gtggccaaga tgcgatgtat 540
gagtatatgg ctcaagcctg tgcaggaaat ggaagtggaa aatcagcagg tagctcattg 600
tcatgcataa atcttgattg ggatgtcata agggataaaa ctaagacaaa gatagagtct 660
ttgaaagagc atggccctat caaaaataaa atgagcgaaa gtcccaataa aacagtatct 720
gaggaaaaag ctaaacaata cctagaagaa tttcatcaaa cggcattaga gcatcctgaa 780
ttgtcagaac ttaaaaccgt tactgggacc aatcctgtat tcgctggggc taactatgcg 840
gcgtgggcag taaacgttgc gcaagttatc gatagcgaaa cagctgataa tttggaaaag 900
acaactgctg ctctttcgat acttcctggg atcggtagcg taatgggcat tgcagacggg 960
gccgttcacc acaatacaga agagatagtg gcacaatcaa tagctttatc gtctttaatg 1020
gttgctcaag ctattccatt ggtaggagag ctagttgata ttggtttcgc tgcataata 1080
ttttagagaa gtattatcaa tttatttcaa gtagttcata attcgtataa tcgtcccgcg 1140
tattctcccg ggcataaaaac gaggcctcat atggcaccta cttcaagttc tacaagaaa 1200
acacagctac aactggagca tttactgctg gatttacaga tgattttgaa tggaaatta 1260
aattacaaga atcccaact caccaggatg ctcacattta agttttacat gcccaagaag 1320
gccacagaac tgaaacatct tcagtgtcta gaagaagaac tcaaacctct ggaggaagt 1380
ctaaatttag ctcaaagcaa aaactttcac ttaagacca gggacttaat cagcaatatt 1440
aacgtaatag ttctggaact aaagggatct gaaacaacat tcattgtgtg atatgctgat 1500
gagacagcaa ccattgtaga atttctgaac agatggatta ctttttgtca aagcatcatt 1560
tcaacactga cttga 1575

```

<210> 14
 <211> 22
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:universal 5' T7
 promoter primer (5' primer for DT constructs)

 <400> 14
 gtaatacgac tcactatagg gc 22

 <210> 15
 <211> 61
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:U2 3' mutagenic
 PCR primer for U2 constructs

 <400> 15
 gatttatgca tgacaatgag ctacctgctg atcttccact tccatttcct gcacaggctt 60
 g 61

 <210> 16
 <211> 61
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:U3 3' mutagenic
 PCR primer for U3 constructs

 <400> 16
 gatttatgca tgacaatgag ctacctgctg attttccact tccatttcct gcacaggctt 60
 g 61

 <210> 17
 <211> 67
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:L1 3' mutagenic
 PCR primer for L1 constructs

 <400> 17
 gatttatgca tgacaatgag ctaccttgac tcaacattcc taatgggtcca tttcctgcac 60
 aggcttg 67

 <210> 18
 <211> 67
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:L2 3' mutagenic
 PCR primer for L2 constructs

<400> 18
 gatttatgca tgacaatgag ctaccttggtg cccataatcc taatgggtcca tttcctgcac 60
 aggcttg 67

<210> 19
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:matrix
 metalloproteinase (MMP) recognition cleavage site,
 MMP substrate octapeptide for L1 constructs

<400> 19
 Gly Pro Leu Gly Met Leu Ser Gln
 1 5

<210> 20
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:matrix
 metalloproteinase (MMP) recognition cleavage site,
 MMP substrate octapeptide for L2 constructs

<400> 20
 Gly Pro Leu Gly Leu Trp Ala Gln
 1 5

<210> 21
 <211> 6
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:urokinase
 plasminogen activator (uPA) recognition cleavage
 site, uPA favorite sequence, uPA substrate
 hexapeptide for U2 constructs

<400> 21
 Gly Ser Gly Arg Ser Ala
 1 5

<210> 22
 <211> 6
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:urokinase
 plasminogen activator (uPA) recognition cleavage
 site, uPA favorite sequence, uPA substrate
 hexapeptide for U3 constructs

<400> 22
 Gly Ser Gly Lys Ser Ala
 1 5

<210> 23
 <211> 6
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:tissue-type
 plasminogen activator (tPA) recognition cleavage
 site, tPA favorite sequence

<400> 23
 Gln Arg Gly Arg Ser Ala
 1 5

<210> 24
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:DTGM-WT furin
 sensitive surface loop sequence

<400> 24
 Cys Ala Gly Asn Arg Val Arg Arg Ser Val Gly Ser Ser Leu Ser Cys
 1 5 10 15

<210> 25
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:DTGM-U2 surface
 loop sequence cleaved by urokinase-type
 plasminogen activator (uPA)

<400> 25
 Cys Ala Gly Asn Gly Ser Gly Arg Ser Ala Gly Ser Ser Leu Ser Cys
 1 5 10 15

<210> 26
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:DTGM-U3 surface
 loop sequence cleaved by urokinase-type
 plasminogen activator (uPA)

<400> 26
 Cys Ala Gly Asn Gly Ser Gly Lys Ser Ala Gly Ser Ser Leu Ser Cys
 1 5 10 15

<210> 27
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:DTGM-L1 surface
 loop sequence cleaved by matrix metalloproteinase
 (MMP)

<400> 27
 Cys Ala Gly Asn Gly Pro Leu Gly Met Leu Ser Gln Gly Ser Ser Leu
 1 5 10 15

Ser Cys

<210> 28
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:DTGM-Fu surface
 loop sequence cleaved only by furin

<400> 28
 Cys Ala Gly Asn Arg Ala Ala Arg Ser Val Gly Ser Ser Leu Ser Cys
 1 5 10 15

<210> 29
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:plasminogen
 activator cleavage site, uPA and tPA physiological
 substrate sequence

<400> 29
 Pro Cys Pro Gly Arg Val Val Gly Gly
 1 5

<210> 30
 <211> 6
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Diphtheria
toxin (DT) cleavage sequence amino acids 163-170

<400> 30

Arg Val Arg Arg Ser Val
1 5